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# Temporal stability of multiple response systems to 7.5% carbon dioxide challenge

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ABSTRACT

Self-reported anxiety, and potentially physiological response, to maintained inhalation of carbon dioxide ($CO_2$) enriched air shows promise as a putative marker of panic reactivity and vulnerability. Temporal stability of response systems during low-dose, steady-state $CO_2$ breathing challenge is lacking. Outcomes on multiple levels were measured two times, one week apart, in 93 individuals. Stability was highest during the $CO_2$ breathing phase compared to pre-$CO_2$ and recovery phases, with anxiety ratings, respiratory rate, skin conductance level, and heart rate demonstrating good to excellent temporal stability (ICCs $\geq$ 0.71). Cognitive symptoms tied to panic were somewhat less stable (ICC = 0.58) than physical symptoms (ICC = 0.74) during $CO_2$ breathing. Escape/avoidance behaviors and *DSM-5* panic attacks were not stable. Large effect sizes between task phases also were observed. Overall, results suggest good-excellent levels of temporal stability for multiple outcomes during respiratory stimulation via 7.5% $CO_2$.

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Carbon dioxide ($CO_2$) hypersensitivity has been studied among persons with panic disorder and other psychiatric conditions, as well as in the general population, and appears to represent a fairly well validated measure of panic reactivity and vulnerability (Battaglia et al., 2007; Coryell, 1997; Coryell, Fyer, Pine, Martinez, & Arndt, 2001; Griez, de Loof, Pols, Zandbergen, & Lousberg, 1990; Perna, Cocchi, Bertani, Arancio, & Bellodi, 1995; Perna, Cocchi, Allevi, Bussi, & Bellodi, 1999). Studies using $CO_2$ enriched air have relied on a variety of $CO_2$ air concentrations, generally ranging from 5% to 35% $CO_2$. Of the concentrations used in clinical research, 35% $CO_2$ is the most commonly used $CO_2$ challenge intensity, likely owing to its procedural ease, wherein participants inhale one or two vital capacity breaths and their anxiety and symptomatic response pre- and post-inhalation are assessed (Amaral et al., 2013). By contrast, the lower $CO_2$ concentrations (e.g., 5%, 7.5%) allow researchers to continuously measure autonomic levels (e.g., respiratory rate, heart rate) as well as self-report of anxiety and symptomatic response, permitting the modeling of change over time.

The National Institute for Mental Health (NIMH) recently launched the Research Domain Criteria (RDoC) project as part of their strategic plan to develop novel ways of classifying psychiatric disorders based on dimensions of observable behaviors and brain functions (Cuthbert & Insel, 2010, 2013; Insel & Cuthbert, 2009; Insel et al., 2010; Sanislow et al., 2010). RDoC aims to serve as a framework for new approaches to research on mental disorders using fundamental dimensions that cut across traditional disorder categories. It is hoped that these fundamental dimensions will closely align with mechanisms that underlie psychopathology at various biological and behavioral levels. The $CO_2$ challenge fits well within the RDoC matrix as a "paradigm" under the Negative Valence Systems and provides for a number of units of analysis, including (but not limited to) physiology, behavior, neural circuits, and self-report.

Given this conceptual shift from diagnostic level phenotypes to dimensional construct measures that fall under higher order diagnoses, the need for psychometrically substantiated measures is crucial. Moreover, given that sensitivity to $CO_2$ is hypothesized to

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be a biologically based trait marker of panic vulnerability (Battaglia et al., 2007; Coryell, 1997; Coryell et al., 2001; Griez et al., 1990; Perna et al., 1995, 1999), it is important to determine whether this marker is consistently expressed across time. Two previous studies examined the test-retest reliability of self-reported anxiety and symptom response to inhalation of 35% $CO_2$, but these studies relied on a small, clinical sample (Verburg, de Leeuw, Pols, & Griez, 1997) and a high- versus low-risk sample (Coryell & Arndt, 1999). Both studies documented reasonable reliability of symptomatic response, particularly smothering sensations, faintness, and dizziness, as well as greater reliability for anxiety rated post versus prior to 35% $CO_2$ inhalation. Physiological and behavioral markers were not measured. Using a within-session repeated measures design, participants' cardiac, electrodermal, and self-report of anxiety were measured in response to eight, 20 s inhalations of 20% $CO_2$ enriched air (Forsyth, Eifert, & Canna, 2000). Means testing indicated no significant trial effects, reflecting stability of measures within session. Collectively these studies suggest satisfactory stability of physiology and subjective response systems to high dose $CO_2$ enriched air. Thus, although the stability of anxiety, symptom, and physiological response to high dose $CO_2$ has been established, a temporal stability study of low dose, steady-state $CO_2$ breathing has not been undertaken.

The objective of the current study is to determine the test-retest reliability of self-reported anxiety and panic symptom response as well as behavioral and physiological markers during the steady-state 7.5% $CO_2$ challenge across two time points. Study outcomes will inform future research and clinical assessments where stable markers are needed. Moderate levels of stability of subjective and symptom report is expected based on previous studies examining stability of response to higher dose $CO_2$ concentrations (Coryell & Arndt, 1999; Forsyth et al., 2000; Verburg et al., 1997). Extant studies of physiological reliability suggest reasonably good temporal consistency (Forsyth et al., 2000; Schmidt et al., 2002). Thus, satisfactory reliability estimates also are expected for physiological outcomes.

## 1. Methods

### 1.1. Participants

Participants were recruited as part of a larger study of $CO_2$ hypersensitivity (n = 376) (Roberson-Nay, Beadel, Gorlin, Latendresse, & Teachman, 2015) at two universities from either the psychology department participant pool or recruitment fliers posted on the campus. Table 1 provides demographic and panic related characteristics for the full test-retest reliability sample (n = 93) as well as for each site. A power calculation conducted before the study's start, indicated a sample size of 86 participants for reliability analysis (see Methods section for full calculation). Thus, the first, consecutive 146 participants completing Session 1 of the $CO_2$ hypersensitivity study at Sites 1 and 2 were invited back to participate in Session 2 of the reliability study. Of the 146 participants invited back for Session 2, 93 (64%) agreed to return. We, therefore, slightly exceeded our power calculation estimation of 86 individuals for reliability analysis.

Study participants at both sites were compensated financially or with course credit; the majority (89%) received course credit. Site 2 recruited participants based on a participant's Anxiety Sensitivity Index (ASI; Reiss, Peterson, Gursky, & McNally, 1986) score, which was completed during a pre-screening session. Participants were recruited based on ASI scores to ensure variance on this important panic and $CO_2$ response risk factor (Blechert, Wilhelm, Meuret, Wilhelm, & Roth, 2013; Korte & Schmidt, 2012; Schmidt, Lerew, & Jackson, 1997, Schmidt, Lerew, & Jackson, 1999; Schmidt, Zvolensky, & Maner, 2006). Thus, Site 2 sent recruitment e-mails to approximately equivalent numbers of students scoring within each quartile of the distribution of ASI scores (Peterson & Reiss, 1992). No pre-screen ASI selection criteria was used for participants who participated for financial compensation or who participated at Site 1; Site 1 was unable to include a pre-screening ASI screen given differences in the participant pool infrastructure. Nonetheless, ASI score distributions and means/variance did not differ between sites (see Table 1). ASI scores also did not differ between participants participating for course credit versus financial compensation ($t(1.89) = 0.95$, $p = 0.34$, Cohen's $d = 0.33$).

Of the 146 participants invited to return for session 2, there were no differences between participants who did and did not return to participate in Session 2 on the following measures, which were assessed at Session 1: repeated measurements of anxiety ratings, Diagnostic Symptom Questionnaire scores, and all physiological measures assessed during the three phases of the $CO_2$ challenge, as well as the Anxiety Sensitivity Index (all $p$'s > 0.05). The one exception was a non-significant trend ($t(1142) = 0.85$, $p = 0.40$, Cohen's $d$=0.15) for the anxiety rating measured during the attachment of the facemask while breathing room air; participants who returned to partake in Session 2 had a slightly higher mean SUDS rating ($M = 23.6$) compared to participants who did not return ($M = 19.5$). There also was no difference in the number of females/males ($\chi^2 = 0.012$, $p = 0.91$) who did and did not return for the Session 2 assessment. Finally, rate of early termination of the $CO_2$ challenge at Session 1 was nearly identical for those participants who agreed to return (21.5%) versus did not agree to return (21.6%) to participate in Session 2 ($\chi^2 = 0.00$ $p = 0.99$). These results suggest that there were not significant differences between participants who did versus did not return to participate in the Session 2 $CO_2$ challenge on primary study outcomes.

To maintain consistency across the test and retest sessions, all Session 2 visits were conducted in the same room as Session 1. Although efforts were made to have the same study team member complete Session 1 and Session 2 with the same test-retest reliability participant, this was not always possible. In total, however,

**Table 1**
Demographic and panic related characteristics measured before 7.5% $CO_2$ challenge for the full reliability sample and by study site.

| | Full Sample n = 93 | Site 1 n = 52 | Site 2 n = 41 | $t/\chi^2$ | $p$ |
|---|---|---|---|---|---|
| Age | 19.9 (4.2) | 20.3 (5.2) | 19.3 (2.3) | 1.1 | 0.29 |
| Sex, % female | 52.7 | 62.7 | 48.6 | 1.7 | 0.19 |
| Self-reported Race, % | | | | | |
| African American | 22.0 | 30.0 | 9.1 | 5.5 | 0.14 |
| Asian | 14.6 | 12.2 | 18.2 | | |
| Caucasian | 57.3 | 51.0 | 66.7 | | |
| Other/More than one Race | 6.1 | 6.1 | 6.1 | | |
| Self-reported Ethnicity, % Hispanic | 12.8 | 9.8 | 17.1 | 1.0 | 0.32 |
| PDSS, % above screening cut-off score ≥ 8 | 14.0 | 17.6 | 8.6 | 0.94 | 0.23 |
| ASI Total Score | 18.8 (10.2) | 18.7 (9.1) | 18.9 | −0.05 | 0.96 |

PDSS = Panic Disorder Severity Scale; ASI = Anxiety Sensitivity Index.

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